CHROMOSOME STUDIES ON *HYDROMYSTRIA LAEVIGATA* (HYDROCHARITACEAE)[1]

EDUARDO A. MOSCONE AND LUIS M. BERNARDELLO[2]

ABSTRACT

Chromosomes of individuals from two Argentine populations of *Hydromystria laevigata* (Willd.) A. T. Hunz. were analyzed for numbers, meiotic behavior, and karyotype. It has $n = 14$ and $2n = 28$. Both populations have regular meiosis and show no significant statistical differences between them. The karyotype is similar in both populations: asymmetrical and bimodal; it is formed by: 4 *m* pairs + 6 *sm* pairs + 4 *st* pairs. No satellites were found.

Hydromystria is a monotypic genus of Hydrocharitaceae. The only species, *H. laevigata,* is an aquatic plant that grows in Mexico, West Indies, and South America (Hunziker, 1981, 1982). Some authors include *Hydromystria* within *Limnobium* (Dandy, 1959; Cook et al., 1974; Cook & Urmi-König, 1983), while others recognize them as distinct monotypic genera although closely related (cf. Hunziker, 1981, 1982).

No comprehensive cytological study has been carried out on *H. laevigata,* although some chromosome counts have been published. Geitler (1938; sub *Trianea bogotensis* Karst.) reported $2n = 26$–28, and the same author (1940) found $4n = 56$ in counts on colchicine-induced tetraploid root cells. Recently, Urmi-König (in Cook & Urmi-König, 1983) reported five different chromosome numbers for this plant [sub *Limnobium laevigatum* (Willd.) Heine]: $2n = 26, 27, 28, 29, 30$. In the present contribution, the somatic and gametic numbers of two Argentine populations of *H. laevigata* are reported, as well as their meiotic behavior and karyotype.

MATERIALS AND METHODS

The materials came from two morphologically identical populations (hereafter named P_1 and P_2) collected in the following localities: Argentina, Córdoba: P_1 = Punilla: Cabalango, *Bernardello, Weigel & Moscone 359 bis,* 29 Aug. 1982; P_2 = Calamuchita: Emblase Río III, camino a la segunda Usina, *Moscone, Carrizo & Moscone 1,* 24 Oct. 1982; we analyzed 35 plants of P_1 and 16 of P_2. Voucher specimens have been deposited in the herbarium of Museo Botánico de Córdoba, Argentina (CORD).

Mitotic chromosomes were examined in root tip squashes; the root tips were fixed in 1 : 3 acetic acid : ethanol mixture after pretreatment in a saturated solution of p-dichlorobenzene in water for 5–7 hours at room temperature, and then stained with alcoholic hydrochloric acid carmine (Snow, 1963) for at least 12 hours. Karyograms were prepared from microphotographs, using the terminology introduced by Levan et al. (1964). Two parameters were used: the arm ratio and the centromeric index. The chromosomes were first arranged according to their increasing arm ratio and then according to the decreasing order within each group. The idiogram is based on ten metaphase plates (five from each population).

Meiotic behavior was observed in pollen mother cells obtained by squashing young anthers fixed in 1 : 3 acetic acid : ethanol and stained with acetic carmine. All the permanent mounts were made with Bradley's method (1948). The statistical methods followed Sokal and Rohlf (1979); as regards the *t*-test, the levels of significance considered were 0.05 and 0.01.

RESULTS

Meiotic observations. Table 1 summarizes the data obtained from the analysis of meiotic behavior. Both populations have regular meiosis, forming usually 14 bivalents (Fig. 1A, B). Sometimes univalents were observed: a 13 II + 2 I configuration was detected in 2.2% of P_1 cells and in up to 3.3% of P_2 cells.

[1] The authors thank Prof. A. T. Hunziker for suggesting the problem and for his continuous support, Dr. J. H. Hunziker for the critical reading of the manuscript, Dr. A. E. Cocucci and Mr. R. Münch for technical assistance. This work was supported in part by a grant from the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba, Argentina. This paper was presented on 22 Sept. 1983 in the XIX Jornadas Argentinas de Botánica organized by the Sociedad Argentina de Botánica at Santa Fe.

[2] Instituto Multidisciplinario de Biología Vegetal (IMBIV), Casilla de Correo 495, 5000 Córdoba, Argentina.

TABLE 1. Meiotic behavior of *Hydromystria laevigata.*

Population	Chromosome Number (n)	Chromosomal Associations in Diplotene-Diakinesis per Cell: Range and Mean ± Standard Error					Mean of Chiasmata per Chromosome Pair: Mean ± Standard Error	Number of Studied Cells
		II	I	Ring II	Chiasmata Total	Terminal Chiasmata		
P_1	14	13–14 13.98 ±0.022	0–2 0.044 ±0.044	1–9 4.73 ±0.295	16–25 20.78 ±0.365	10–19 15.58 ±0.319	1.48 ±0.026	45
P_2	14	13–14 13.97 ±0.019	0–2 0.065 ±0.037	1–8 4.53 ±0.168	15–25 20.83 ±0.226	7–20 15.15 ±0.260	1.49 ±0.016	92
t-test		$t_{135} = 0.32$ $P \cong 0.7$	$t_{135} = 0.34$ $P \cong 0.7$	$t_{135} = 0.63$ $P \cong 0.5$	$t_{135} = 0.12$ $P \cong 0.9$	$t_{135} = 0.99$ $P \cong 0.3$	$t_{135} = 0.34$ $P \cong 0.7$	

FIGURE 1. *Hydromystria laevigata.*—A. Diakinesis, 14 II (*Moscone et al. 1*).—B. Diakinesis, 14 II (*Bernardello et al. 359 bis*).—C. First mitotic metaphase of pollen grain, $n = 14$ (*Moscone et al. 1*).

Two bivalents much bigger than the others have from two to six chiasmata; they correspond to pairs 1 and 11 from the karyotype, although it is not always possible to distinguish one pair from the other. The remaining bivalents are shorter, similar to each other in length, and generally have only one terminal chiasma. Sometimes they sustain two terminal chiasmata, thus forming ring bivalents. Occasionally, the association of some bivalents to the nucleolus and the first mitotic division of the microspore were observed (Fig. 1C).

Mitotic observations. The somatic chromosome number $2n = 28$ (Fig. 2) was found in 53 metaphase plates of P_1 and 22 of P_2. Based on their arm ratio, the chromosomes can be classified as follows (Fig. 3A, B): 4 *m* pairs (1 to 4), 6 *sm* pairs (5 to 10) and 4 *st* pairs (11 to 14). It should be emphasized that no chromosome is satellited, at least according to our observations. The karyotype is asymmetrical and bimodal.

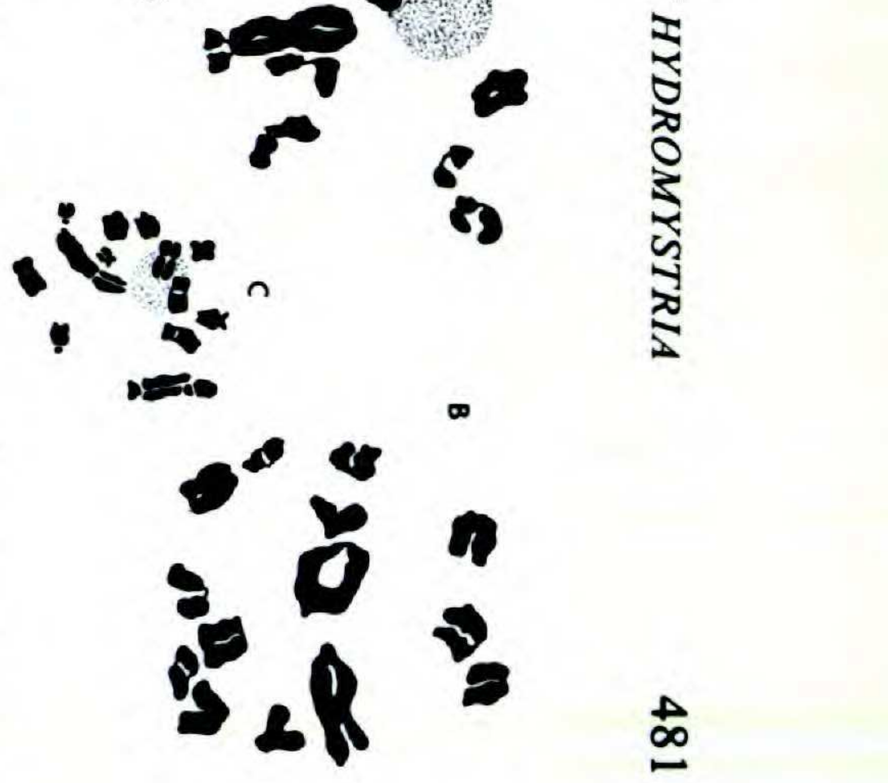

FIGURE 2. Mitotic metaphases of *Hydromystria laevigata*, $2n = 28$.—A. *Moscone et al. 1.*—B. *Bernardello et al. 359 bis.*

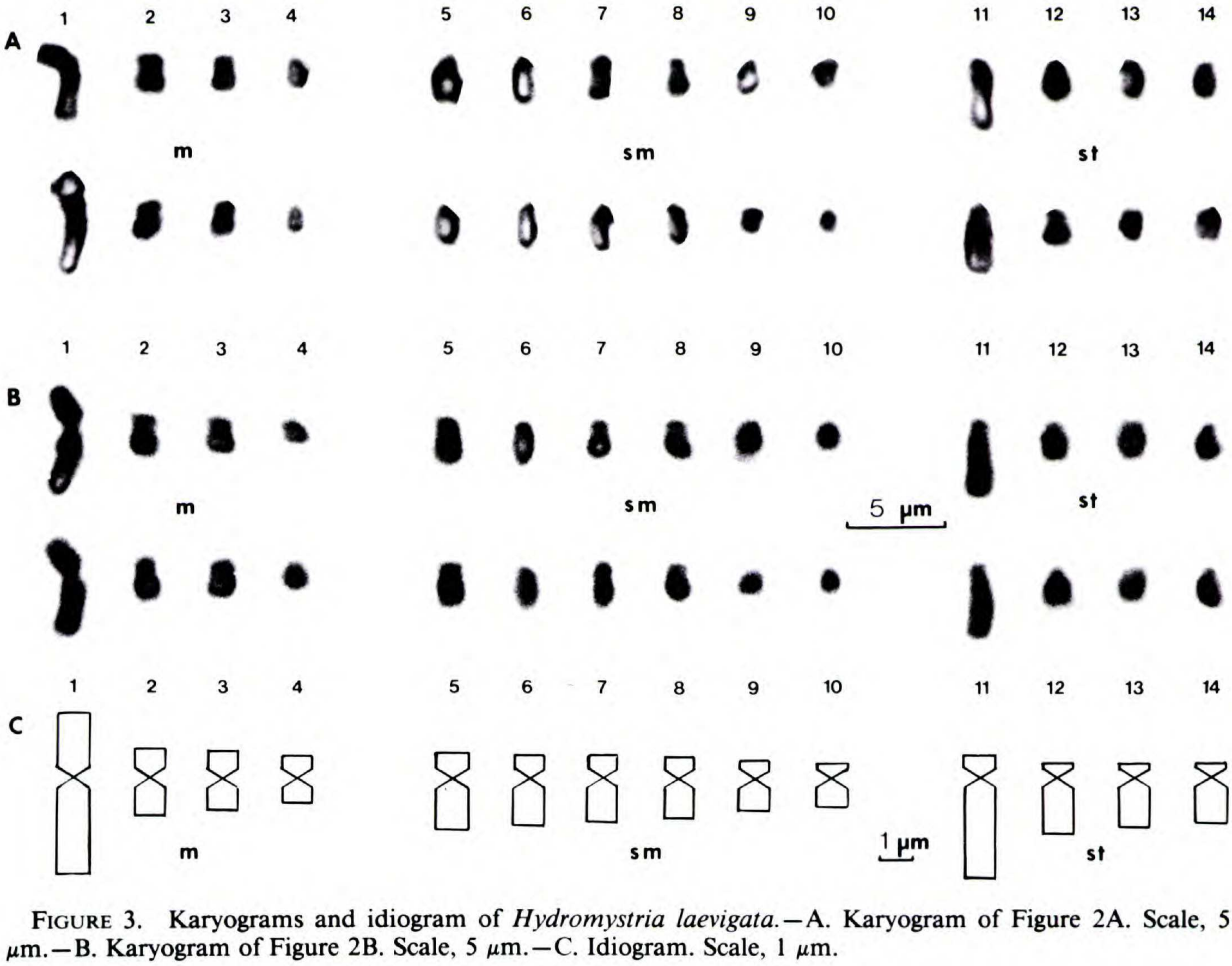

FIGURE 3. Karyograms and idiogram of *Hydromystria laevigata*.—A. Karyogram of Figure 2A. Scale, 5 µm.—B. Karyogram of Figure 2B. Scale, 5 µm.—C. Idiogram. Scale, 1 µm.

Pairs 1 and 11 are considerably larger than the others, which are in general homogeneous in size and thus difficult to characterize. The idiogram (Fig. 3C) was based on chromosome length, data for which are presented in Table 2. Pair 1 is the longest, and pair 10 is the shortest. The lowest arm ratio corresponds to pair 4 and the highest one to pair 11.

DISCUSSION

From the meiotic behavior and the *t*-test results it is evident that both populations have regular meiosis and show no statistical differences in their meiotic systems. Furthermore, the analysis of mitotic chromosomes demonstrates a striking similarity between P_1 and P_2 karyograms although Chaudhuri and Sharma (1978) have mentioned, based on their investigations on *Vallisneria spiralis* L., *Ottelia alismoides* Pers., *Hydrilla verticillata* (L. f.) Royle, and others, the frequent presence of cytotypes in Helobiales. These authors found several cytotypes of those species varying in both chromosome number and morphology. This circumstance has been reported in other members of the Hydrocharitaceae by other botanists (Bhattacharya & Gosh, 1976; Harada, 1956; Misra, 1974; Sharma & Bhattacharyya, 1956).

In contrast to our results, Urmi-König (in Cook & Urmi-König, 1983), studying plants from Guadeloupe, Buenos Aires, and of unknown origin, indicated $2n = 26, 27, 28, 29, 30$ for *H. laevigata* with several chromosome numbers in an individual specimen. Although some differences can be drawn from her karyogram data, it is difficult to compare them because she did not state the terminology used and did not show any figure or photograph. She found one pair of long submetacentric and one medium pair designed as acrocentric. After the widely accepted terminology of Levan et al. (1964), both long pairs are *m* and *sm* according to our results. Urmi-König did not report *sm* chromosomes within the short pairs but did report a number of acrocentric ones.

Geitler (1938) could find neither satellites nor

TABLE 2. Measurements and indexes of somatic chromosomes of *Hydromystria laevigata*. Measurements given in μm. Abbreviations after Levan et al. (1964).

Pair	Chromosome Lengths: Range and Mean ± Standard Error			*r*	i	Nomenclature
	s	l	c			
1	1.72–2.55 2.00 ± 0.092	2.55–4.00 3.06 ± 0.152	4.33–6.55 5.06 ± 0.243	1.53	39.53	m
2	0.80–1.05 0.92 ± 0.028	1.05–1.45 1.21 ± 0.046	1.90–2.50 2.13 ± 0.070	1.32	43.19	m
3	0.73–1.07 0.86 ± 0.036	0.90–1.38 1.09 ± 0.052	1.70–2.45 1.95 ± 0.083	1.27	44.10	m
4	0.60–0.80 0.67 ± 0.019	0.70–0.95 0.77 ± 0.024	1.30–1.75 1.44 ± 0.042	1.15	46.53	m
5	0.68–0.90 0.77 ± 0.024	1.35–2.05 1.61 ± 0.065	2.08–2.90 2.38 ± 0.081	2.09	32.35	sm
6	0.60–0.92 0.69 ± 0.030	1.28–1.83 1.50 ± 0.056	1.90–2.75 2.19 ± 0.078	2.17	31.51	sm
7	0.60–0.80 0.69 ± 0.020	1.15–1.65 1.36 ± 0.051	1.80–2.45 2.05 ± 0.066	1.97	33.66	sm
8	0.57–0.85 0.69 ± 0.027	1.02–1.50 1.22 ± 0.046	1.60–2.35 1.91 ± 0.073	1.77	36.13	sm
9	0.45–0.65 0.52 ± 0.019	0.83–1.15 0.99 ± 0.027	1.30–1.80 1.51 ± 0.043	1.90	34.44	sm
10	0.40–0.58 0.45 ± 0.018	0.80–1.10 0.91 ± 0.029	1.20–1.68 1.36 ± 0.044	2.02	33.09	sm
11	0.60–0.90 0.70 ± 0.030	2.65–4.20 3.10 ± 0.144	3.30–5.10 3.80 ± 0.172	4.43	18.42	st
12	0.35–0.50 0.43 ± 0.019	1.35–2.10 1.63 ± 0.067	1.70–2.60 2.06 ± 0.082	3.79	20.87	st
13	0.37–0.60 0.45 ± 0.021	1.23–1.90 1.49 ± 0.062	1.60–2.50 1.94 ± 0.082	3.31	23.20	st
14	0.37–0.52 0.43 ± 0.016	1.20–1.70 1.39 ± 0.053	1.60–2.20 1.82 ± 0.068	3.23	23.63	st

secondary constrictions in this species. We agree with his observations, although Urmi-König (in Cook & Urmi-König, 1983) reported that one pair bears satellites but without specifying which pair.

Like other Hydrocharitaceae examined (Misra, 1974; Chaudhuri & Sharma, 1978; Bhattacharya & Gosh, 1976; Sharma & Bhattacharyya, 1956) *Hydromystria* has an asymmetrical karyotype.

The basic number of $x = 7$ for *H. laevigata* is one of several previously recorded for the family. The basic numbers, in decreasing order of frequency, are: $x = 8$, 11, 10, 7, and 6 (Fedorov, 1969; Goldblatt, 1981, 1984; Moore, 1973, 1977; Packer & Witkus, 1982).

Some of the genera of Hydrocharitaceae have not been studied cytologically: *Apalanthe, Appertiella, Limnobium* sensu stricto, and *Thalassia,* and the genera *Nechamandra* and *Stratiotes* are insufficiently known. A cytological study of *Limnobium* may help elucidate its relation to *Hydromystria,* on which we are currently working.

LITERATURE CITED

BHATTACHARYA, G. N. & D. K. GOSH. 1976. Cytological analysis of different cytotypes of *Ottelia alismoides* Pers. Caryologia 29: 195–202.

BRADLEY, M. V. 1948. A method for making acetocarmine squashes permanent without removal of cover slip. Stain Technol. 23: 41–44.

CHAUDHURI, J. B. & A. SHARMA. 1978. Cytological studies on three aquatic members of Hydrocharitaceae in relation to their morphological and ecological characteristics. Cytologia 43: 1–19.

COOK, C. D. K. & K. URMI-KÖNIG. 1983. A revision of the genus *Limnobium* including *Hydromystria* (Hydrocharitaceae). Aquatic Bot. 17: 1–27.

———, B. J. GUT, E. M. RIX, J. SCHNELLER & M. SEITZ. 1974. Water Plants of the World. Dr. W. Junk Publ., The Hague.

DANDY, J. E. 1959. Hydrocharitaceae (keys for the tribes and genera). Pp. 540–541 *in* J. Hutchinson (editor), The Families of Flowering Plants. Volume 2. Clarendon Press, Oxford.

FEDOROV, A. (editor). 1969. Chromosome Numbers of Flowering Plants. V. L. Komarov Bot. Inst., Leningrad. [Reprinted by O. Koeltz Sci. Publ., Koeningstein. 1974.]

GEITLER, L. 1938. Über das Wachstum von Chromozentrenkernen und zweierlei Heterochromatin bei Blütenpflanzen. Z. Zellf. Mikroskop. Anat., Abt. B, Chromosoma 28: 133–153.

———. 1940. Die Polyploidie der Dauergewebe höherer Pflanzen. Ber. Deutsch. Bot. Ges. 58: 131–142.

GOLDBLATT, P. (editor). 1981. Index to plant chromosome numbers 1975–1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5: 279.

———. 1984. Index to plant chromosome numbers 1979–1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8: 203.

HARADA, I. 1956. Cytological studies in Helobiae, I. Chromosome idiograms and a list of chromosome numbers in seven families. Cytologia 21: 306–328.

HUNZIKER, A. T. 1981. *Hydromystria laevigata* (Hydrocharitaceae) en el centro de Argentina. Lorentzia 4: 5–8.

———. 1982. Observaciones biológicas y taxonómicas sobre *Hydromystria laevigata* (Hydrocharitaceae). Taxon 31: 472–477.

LEVAN, A., K. FREDGA & A. SANDBERG. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220.

MISRA, M. P. 1974. Cytological studies in *Ottelia alismoides* Pers. Cytologia 39: 419–427.

MOORE, R. J. (editor). 1973. Index to plant chromosome numbers for 1967–71. Regnum Veg. 90: 50.

———. 1977. Index to plant chromosome numbers for 1973–74. Regnum Veg. 96: 124.

PACKER, J. G. & R. WITKUS. 1982. Hydrocharitaceae. *In* A. Löve (editor), IOPB Chromosome Number Reports LXXV. Taxon 31: 363.

SHARMA, A. K. & B. BHATTACHARYYA. 1956. A study of the cytology of four members of the Hydrocharitaceae as an aid to trace the lines of evolution. Phyton (Buenos Aires) 6: 121–132.

SNOW, R. 1963. Alcoholic hydrochloric acid-carmine as stain for chromosomes in squash preparations. Stain Technol. 38: 9–13.

SOKAL, R. R. & F. J. ROHLF. 1979. Biometría. H. Blume Ediciones, Madrid. [Translated from the 1969 English 1st edition.]